CASE REPORT

Acute Hemolysis Caused by Incidental Trichlorfon Exposure

Ming-Ling Wu*, Jou-Fang Deng

Division of Clinical Toxicology, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.

Trichlorfon (o-o-dimethyl-2,2,2-trichloro-hydroxyethylphosphate), an organophosphate, has a moderately potent anticholinesterase activity. Organophosphate poisoning is well known for its characteristic symptoms and signs, but acute hemolysis caused by trichlorfon is rarely reported. We present a patient who developed acute hemolysis and renal function impairment after percutaneous trichlorfon exposure. A 54-year-old man applied trichlorfon powder to his dog to kill its parasites. Half an hour later, the dog was suspected to die of cholinergic crisis and the patient felt abdominal cramping pain. Later, he developed severe nausea, vomiting, chills, high fever, and cold sweat. Laboratory work-up disclosed a picture of acute hemolysis, jaundice, renal function impairment and leukocytosis. However, there were no clinical features of acute cholinergic syndrome except gastrointestinal symptoms, and blood cholinesterase activities were also normal. He eventually had a full recovery. Trichlorfon should be added to the toxins known to cause acute hemolysis. [*J Chin Med Assoc* 2009;72(4):214–218]

Key Words: acute hemolysis, acute trichlorfon poisoning, percutaneous exposure

Introduction

Trichlorfon (o-o-dimethyl-2,2,2-trichloro-hydroxyethylphosphate), an organophosphate, is used as an insecticide in crop protection. In domestic animals, trichlorfon is also used for the control of external and internal parasites. The synonyms of trichlorfon include chlorofos and dipterex. It is a water-soluble organophosphate insecticide with a moderately potent anticholinesterase activity. The acute median lethal dose (LD_{50}) in rats is 450–650 mg/kg (oral) and 2,000–5,000 mg/kg (skin).[1] As with all organophosphates, trichlorfon is readily absorbed through the skin and is subsequently excreted in urine. Skin sensitivity (allergies) can result from dermal exposure.

Organophosphate pesticides are widely used in Taiwan, and the incidence of organophosphate poisoning is high.[2] Organophosphate decreases activity of the cholinesterase enzyme which is necessary for normal nervous system function. Organophosphate poisoning is characterized by typical manifestations of cholinergic excess. Signs and symptoms of acute exposure can be divided into 3 broad categories. Muscarinic effects include bradycardia, bronchospasm, bronchorrhea, salivation, lacrimation, diaphoresis, vomiting, diarrhea, urination, and miosis. Nicotinic effects include tachycardia, hypertension, mydriasis, muscle fasciculations, muscle cramps, weakness, and diaphragmatic failure. Central effects include central nervous system depression, headache, giddiness, agitation, confusion, delirium, coma, and seizures. Trichlorfon has been associated with induction of delayed polyneuropathy after large exposures, causing severe cholinergic toxicity.[3]

Like other organophosphates, the clinical presentations of acute trichlorfon poisoning are typical cholinergic syndrome; however, the severity of poisoning is mild compared with that of other organophosphates. We report a case of acute trichlorfon poisoning that had unusual presentations of acute hemolysis and renal function impairment.

Case Report

A 54-year-old man used trichlorfon to kill his dog's parasites. He neglected the instructions of diluting it

*Correspondence to: Dr Ming-Ling Wu, Division of Clinical Toxicology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: mlwu@vghtpe.gov.tw • Received: July 1, 2008 • Accepted: December 23, 2008

1:100 in the belief that it would be more effective not to dilute it. He sprayed some water to wet his dog and then directly applied the powder to its whole body. During the procedure, he did not wear protective gloves. Half an hour later, the dog suffered from salivation, sweating, respiratory distress and death. Simultaneously, the patient developed abdominal cramping pain. This was followed by severe nausea and vomiting 4 hours later, and he went to a nearby hospital for help. The laboratory tests were remarkable for leukocytosis, with white blood cell count (WBC) of 19,500/mm^3 and elevated total bilirubin of 2.2 mg/dL (normal, 0.2–1.6 mg/dL). He was treated with intravenous fluids and discharged. Unfortunately, fever, chills, cold sweats and generalized weakness occurred the next day, so he went back to the hospital. Follow-up tests disclosed marked leukocytosis (WBC, 30,190/mm^3), elevated blood urea nitrogen of 35 mg/dL (normal, 7–20 mg/dL), creatinine of 2.3 mg/dL (normal, 0.5–1.5 mg/dL), and total bilirubin of 5.0 mg/dL. Intravenous fluids and pralidoxime 1 g were administrated. The patient was then referred to our hospital for the unusual presentation of trichlorfon intoxication.

On arrival, he was conscious and complained of dizziness, abdominal pain, chills, and weakness. Prior to this admission, he was healthy except for having hyperuricemia with gouty arthritis and herniation of an intervertebral disc of the lumbar spine which had received operation. His vital signs were blood pressure of 106/60 mmHg, pulse rate of 81/min, respiratory rate of 18/min, and body temperature of 36.6°C. Physical examinations were generally normal, except for slight icteric sclera. His pupils were isocoric (2 mm), with prompt light reflex. His abdomen was not tender, and his liver and spleen were not palpable. Hematologic tests showed leukocytosis (WBC, 33,400/mm^3; 72% polymorphonucleated neutrophils; 13% banded form) with a left shift, hemoglobin of 16.4 g/dL, and platelet count of 159,000/mm^3. Serum biochemical tests revealed blood urea nitrogen of 40 mg/dL, creatinine of 2.1 mg/dL, total bilirubin of 4.75 mg/dL, direct bilirubin of 1.42 mg/dL (normal, 0–0.2 mg/dL), alanine aminotransferase of 36 U/L (normal, 0–40 U/L), aspartate aminotransferase of 35 U/L (normal, 5–45 U/L), alkaline phosphatase of 60 U/L (normal, 10–100 U/L), γ-glutamyl transferase of 185 U/L (normal, 8–61 U/L), lactate dehydrogenase of 239 U/L (normal, 85–213 U/L), and creatine phosphokinase (CPK) of 37 U/L. C-reactive protein was high, at 23.1 mg/dL (normal, 0.5–1.5 mg/dL). Serum amylase and lipase were normal. Serum electrolytes were: sodium 142 mmol/L; potassium 4.6 mmol/L; and chloride 105 mmol/L. Free calcium was decreased to 1.04 mmol/L. Urinalysis showed protein 2+, bilirubin 1+, red blood cells 4–6/high-power field, WBC 0–1/high-power field, and granular cast 2–4/high-power field. The serial laboratory tests are summarized in Table 1. Chest X-ray was normal. Electrocardiography disclosed normal sinus rhythm and left atrium enlargement. Intravenous fluid with electrolyte supplement was then commenced, and he was admitted with supportive care.

During the period of hospitalization, the patient complained of weakness, fever, dull abdominal discomfort, and itching skin rash. The fever was low-grade, with body temperature around 37.3–38.4°C, and persisted about 7 days. Hemoglobin decreased to 14.2 g/dL on day 9. C-reactive protein was elevated to 39.5 mg/dL on the 3rd day post-exposure, which represented the presence of active inflammation. A full evaluation including high plasma hemoglobin of 25.4 mg/dL (normal, 1–5 mg/dL), low haptoglobin < 13 mg/dL (normal, 70–379 mg/dL), positive urine hemosiderin and some hemoglobin drop (2.2 g/dL) confirmed the presence of acute hemolysis. Erythrocyte cholinesterase and plasma cholinesterase activities were 52 μmol/sec/L (normal, 20–72 μmol/sec/L) and 26 μmol/sec/L (normal, 20–70 μmol/sec/L), respectively. Evaluations of other potential etiologies were negative. These tests included both direct and indirect Coombs' tests, erythrocyte osmotic fragility test, autohemolysis test, G6PD assay and antinuclear antibody. Prothrombin time was 0.94 international normalized ratio, and activated partial thromboplastin time was 28.8 seconds, which were within normal limits. Blood culture was negative. Abdominal sonography only disclosed moderate-degree fatty liver. After fluid resuscitation with normal saline, renal function returned to normal, and the patient was discharged on the 10th day.

Follow-up at 15 days post exposure showed mild elevation of liver enzymes. Haptoglobin and plasma hemoglobin returned to 152 mg/dL and 8.31 mg/dL, respectively. Cholinesterase activities remained normal (erythrocyte cholinesterase, 50 μmol/sec/L; plasma cholinesterase, 31 μmol/sec/L). Telephone follow-up at 6 months revealed that the patient had completely recovered without sequelae.

Discussion

Trichlorfon is primarily an indirect inhibitor of acetylcholinesterase; it is converted in the body to the active chemical inhibitor dichlorvos. In fact, trichlorfon is a slow-release cholinesterase inhibitor, transformed

Table 1. Selected laboratory data following percutaneous trichlorfon exposure

	Day 1	Day 2 AM	Day 2 PM	Day 3	Day 4	Day 6	Day 9	Day 15
Leukocytes (4,500–11,000/mm^3)	19,590	30,190	33,400	26,900	16,100	12,700	11,300	15,100
Band/Seg/Lymphocytes			13/72/7	6/92/1	7/83/5	–/80/16	–/72/17	–/72/19
Erythrocytes (4.6–6.2 × 10^6/mm^3)			4.09	4.91	4.68	4.72	4.49	5.06
Hb (14–18 g/dL)			16.4	15.6	14.8	14.8	14.2	15.6
Platelets (150–350 × 10^3/mm^3)			159	143	149	171	315	467
BUN (7–20 mg/dL)	21.8	35	40	43	21	14	15	
Creatinine (0.5–1.5 mg/dL)	0.8	2.3	2.1	1.3	0.8	0.9	0.8	
Total bilirubin (0.2–1.6 mg/dL)	2.2	5.0	4.75	6.14	3.8	1.5	1.0	
Direct bilirubin (0–0.2 mg/dL)			1.42		1.0			
Creatinine phosphokinase (0–140 U/L)			37	23	13			
ALP (10–100 U/L)			60	68	94	150	180	132
γ-glutamyl transferase (8–61 U/L)			185	158	138	208	218	178
LDH (85–213 U/L)					239	259		
ALT (0–40 U/L)			36	41	41	64	64	51
AST (5–45 U/L)			35	34	25	40	35	23
Haptoglobin (30–200 mg/dL)					< 13			152
Plasma Hb (1–5 mg/dL)					25.4			8.31
CRP (0.5–1.5 mg/dL)			23.1	39.5			4.9	
Glucose (65–125 mg/dL)			118	174				
Sodium (135–147 mmol/L)			141		137		138	
Potassium (3.4–4.7 mmol/L)			4.6		3.7		4.8	
Chloride (100–114 mmol/L)			103		105			

Hb = hemoglobin; BUN = blood urea nitrogen; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein.

non-enzymatically to dichlorvos. As a result, signs and symptoms due to trichlorfon overexposure develop after a latent period and may even continue to increase after exposure has been discontinued.[4] Typical presentations of acute trichlorfon poisoning are cholinergic syndrome. In this incident, the dog died rapidly after trichlorfon exposure; the manifestations were compatible with cholinergic crisis. However, the patient did not have symptoms of organophosphate poisoning except gastrointestinal upset (abdominal pain, vomiting) and his cholinesterase activities were within normal ranges. Based on the clinical findings, we precluded that acute renal function impairment and hemolysis were due to trichlorfon exposure. The gastrointestinal symptoms were nonspecific, and they can be part of the effects of free plasma hemoglobin.[5]

In the Chinese literature, Liu et al reported a case of cold antibody autoimmune hemolytic anemia induced by diptrex (trichlorfon).[6] The patient was a 6-year-old Chinese girl who got head lice, so her parents dissolved 5 g diptrex in water, then applied it to her hair. She felt dizziness, fatigue, and intermittent abdominal pain the next day. Jaundice, tea-color urine, and high fever developed thereafter. She was sent to hospital on the 4th day, where hemolytic anemia, positive cold agglutinin, renal and hepatic function impairment were noted. She improved after hospitalization for 11 days. Her hemoglobin was 2.5 g/dL on the first admission day, and elevated to 7.2 g/dL after treatment. This case was diagnosed as cold agglutinin disease induced by trichlorfon. Our case was of an adult Taiwanese who also presented with acute hemolysis and acute renal impairment after percutaneous trichlorfon poisoning, but the hemolysis in our case was non-immune mediated. Apart from these 2 cases, neither trichlorfon nor other organophosphates has been reported to be responsible for acute hemolysis and direct renal damage in poisoning cases.

In cases of organophosphate poisoning, the toxicity of organophosphate insecticides is readily explained by their toxicology. However, the role of solvents or other additives should be considered for the unusual presentation. The trichlorfon powders do not contain a solvent; some contain starches as addictives. Therefore, the toxicity only comes from the insecticide itself.

Organic material is one of the causes of hemolysis. Our patient had positive urine hemosiderin, low haptoglobin, elevated indirect bilirubin, lactate dehydrogenase and plasma hemoglobin, which indicated that the condition was due to intravascular hemolysis.

The possible causes of acquired hemolysis may include entrapment, immune-mediated antibodies, trauma (impact hemolysis and macro- or microvascular defect), paroxysmal nocturnal hemoglobinuria, and toxic effects on erythrocyte membranes (infection or organic material). In these 2 cases, trichlorfon exposure was the most likely cause of the hemolysis. In reviewing the known literature, there are limited data with regard to the effects of organophosphates on hemolysis. Experimentally, organophosphate insecticides have both antihemolytic and hemolytic effects, which depend on different experimental conditions and compounds.

Blasiak et al reported that some organophosphates exert antihemolytic effects on pig erythrocytes by preventing osmotic disruption of the membrane in hypotonic saline media.[7] The tested organophosphates included methylbromphenvinphos, methylparathion, and dichlorvos. They also noted that the antihemolytic effect had reversed correlation with the organophosphate's water solubility. In this experiment, dichlorvos had the weakest antihemolytic effect.

de Potas and de D'Angelo reported a membrane perturbational effect observed in people affected by severe and mild organophosphorus insecticide intoxication.[8] Their results showed that these compounds produce the stabilization of the erythrocytes against hypotonic challenge. Changes in phospholipid distribution were observed and led to hemolysis in severe organophosphate poisoning. They concluded that organophosphates may have a biphasic action on erythrocyte stability according to their concentrations. Higher concentrations produce gross membrane damage with leakage of cellular constituents, while low concentrations are membrane stabilizers. Singh et al studied 4 organophosphate pesticides (dimethoate, chlorpyrifos, ethion and monocrophos) on porcine erythrocytes and found that the 4 pesticides increased hemolysis and potassium leakage from erythrocytes.[9]

The above experiments indicate that high concentrations of trichlorfon may have the potential to induce hemolysis by changes in the phospholipid distribution of erythrocyte membranes. In our patient, the acute hemolysis was confirmed by clinical and laboratory evidence. Further study needs to be done to evaluate the potential effect of trichlorfon on human erythrocyte disruption.

Trichlorfon usually shows no nephrotoxicity in experimental animals and no significant effect on kidney function in humans.[1] Neither trichlorfon nor other organophosphates has ever been reported to be directly responsible for acute renal injury. The nephrotoxicity of organophosphates is often secondary to renal hypoperfusion following circulatory failure. As this patient showed no episodes of shock during his illness, the postulated cause of the renal damaging effect might be the direct toxicity of trichlorfon or a secondary effect of acute hemolysis. Whether or not trichlorfon has a direct nephrotoxic effect on renal tubules is unknown. The differential diagnosis is usually made by histopathologic studies of the kidney and special biochemical studies. In our patient, renal function was impaired within 24 hours after trichlorfon percutaneous absorption and improved in the following days without any specific treatment. Renal biopsy was not performed due to transient and reversible renal injury. The precise pathophysiologic mechanisms are unknown in our patient. One of the mechanisms for renal toxicity is intravascular hemolysis with hemoglobinuria and its consequent nephrotoxicity. Another proposed mechanism is excess plasma hemoglobin increasing nitric oxide scavenging, and depletion of nitric oxide is associated with vasoconstriction and end organ hypoperfusion.[5] Using a canine model, Minneci et al demonstrated that hemolysis produced dose-dependent vasoconstriction and impaired renal function secondary to the stochiometric oxidation of nitric oxide by cell-free plasma oxyhemoglobin.[10]

Trichlorfon also causes toxicity via production of free radicals and impairment of antioxidant systems, in addition to its typical action as an inhibitor of acetylcholinesterase.[11] Our patient did not have the presentation of anticholinesterase poisoning, but he did have acute hemolysis. It is important for physicians to be aware of these unusual presentations of trichlorfon poisoning. The differential diagnosis of intravascular hemolysis should include trichlorfon poisoning.

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